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Occurrence of a Novel DNA Virus (TTV) Infection in Patients With Liver Diseases and Its Frequency in Blood Donors

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A novel DNA virus (TTV) was identified recently in Japanese patients with posttransfusion hepatitis non-A-E and has been implicated as a cause of acute and chronic liver diseases of unknown etiology in some patients. The frequency of TTV infections was investigated in 284 blood donors, 105 patients with different liver disorders before and after liver transplantation (OLT), as well as in 64 patients with chronic hepatitis C who received antiviral therapy. TTV infections were found more frequently by nested-PCR in patients with liver disorders (15%) as compared to blood donors (7%). TTV occurred independently of the aetiology of the liver disease (e.g., cryptogenic cirrhosis [12.5%], alcoholic cirrhosis [16%], fulminant hepatic failure non-A-E [35%], and chronic hepatitis C [12.5%]; p=n.s.). After OLT, a high rate of TTV de novo infections (44%) was observed. However, TTV viremia after OLT (in 56 out of the 105 patients) was not associated with graft hepatitis. Analysis of patients with chronic hepatitis C coinfected with TTV who have been treated with interferon alpha alone or in combination with ribavirin revealed that TTV is an interferon-sensitive virus. Phylogenetic analysis of TTV sequences suggest that at least four different genotypes and several subtypes exist in Germany. In conclusion, the high prevalence of TTV infections observed in patients with parenteral risk factors is an argument in favour of transmission of the virus via blood and blood products. A relevant hepatitis-inducing capacity of TTV, however, seems unlikely, considering the observation that in the majority of patients, TTV infection after OLT was not accompanied by graft hepatitis. ***J. Med. Virol. 59:117–121, 1999.***

KEY WORDS: phylogenetic analysis; graft hepatitis; liver transplantation; cryptogenic cirrhosis; fulminant hepatitis non-A-E

INTRODUCTION

TTV was first identified in the serum of a patient (TT) with posttransfusion non-A to G hepatitis by representational differential analysis and reported to be an unenveloped, single-stranded DNA virus with similarities to the Parvoviridae family [Nishizawa et al., 1997; Okamoto et al., 1998a]. TTV sequences were found in more than 45% of Japanese patients with fulminant hepatic failures or chronic liver disease but also in patients with parenteral risk factors (e.g., hemodialysis patients, hemophiliacs, intravenous drug users) and in 12% of healthy Japanese blood donors [Okamoto et al., 1998a]. It was deduced from these observation that TTV may be another candidate virus, after GB virus C [Simons et al., 1995] or hepatitis G virus [Linnen et al., 1996] for unexplained hepatitis cases. Recent studies from the UK, Thailand, USA, Germany, and Japan confirmed the overall high prevalence of TTV infection in the general population, blood donors, and patients with liver disorders [Simmonds et al., 1998; Naoumov et al., 1998, Tanaka H et al., 1998; Charlton et al., 1998; Höhne et al., 1998; Takahashi et al., 1998]. However, in these studies no clear association could be established between TTV infection and

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clinical manifestations. We investigated the frequency of TTV infections in German patients with different liver diseases and in healthy blood donors. The principle findings are the lack of an association of TTV infection with liver disease and the lower frequency of TTV infection in German blood donors as compared to those in Japan. Furthermore, the TTV sequence data indicate that at least four different TTV genotypes and several subtypes may exist in Germany.

MATERIAL AND METHODS

Two hundred eighty-four adults (106 females, 178 males; mean age 36 (1.4 years, ranging 20–65 years) presenting at the blood bank for their first blood donation from February to June 1996 were examined. Thirty-five (12%) donors had elevated aminotransferase (ALT) levels (mean 50 ± 2.2 U/L; range, 31–83 U/L; [normal ALT values for males < 23 U/L, and for females < 20 U/L]). In the remaining 249 donors ALT levels were within the normal range (mean 11 (±0.7 U/L; range, 5–21 U/L). All donors were negative for antibody to hepatitis C virus (HCV), hepatitis B virus (HBV), and human immunodeficiency virus (HIV)-1 and HIV-2. None of the donors had a history of blood transfusion.

One hundred sixty-nine patients with acute and chronic liver diseases were examined. One hundred five underwent orthotopic liver transplantation (OLT) and serum samples were studied before and after OLT (mean 38 months after OLT; range, 1–90 months). Thirty-two patients (16 males, and 16 females; mean age 45 ± 1.7 years; range, 26–58 years) had decompensated chronic liver disease of unknown origin (cryptogenic cirrhosis). Histological examination of liver biopsies was undertaken in all patients during the follow-up up to 90 months after OLT (mean 38 ± 5.0 months; range, 1–90 months). In total, 131 liver biopsies were investigated (1–10 per patient).

Fifty-six patients (37 males and 19 females; mean age 45 ± 0.9 years; range, 27–59 years) suffered from decompensated alcoholic liver disease. In total, 223 liver biopsies could be examined, 2–8 per patient (mean 4) in a mean follow-up period of 38 ± 2.1 months (range, 1–61 months) after OLT.

Seventeen patients (4 males, 13 females; mean age 34 ± 3.1 years; range, 17–51 years) presented with a non-A-E fulminant hepatic failure. In total, 89 liver biopsies were examined, (mean 5 per patient; range, 2–8) in a mean follow-up of 39 (6.2 months (range, 1-64 months) after OLT.

All patients had received transfusion of blood and blood products during the transplantation procedure. Patients with fulminant hepatic failure had been transfused before OLT and before serum samples for TTV testing were drawn.

The remaining 64 patients suffered from chronic hepatitis C (33 men and 31 women, mean age 44 ± 1.3 years; range, 26–63 years) and had been treated with either recombinant interferon alpha (IFNa) 6 MU three times weekly (n = 31) or IFNa in combination with ribavirin (14 mg/kg/day) (n = 33). Individual serum samples were tested before, during, and after therapy. Stored peripheral blood mononuclear cells (PBMC) of these patients were also examined for the presence of TTV DNA before therapy.

In the detection of TTV sequences, total DNA was extracted from 100 μL serum and buffy coat (10^6 PBMCs) using QIAamp Blood kit (QIAGEN®; Hilden, Germany) and resuspended in 100 μL elution buffer. TTV DNA sequences were amplified by nested polymerase chain reaction (PCR) as described recently [Höhne et al., 1998]. Nested PCR products were sequenced directly using ABI Prism 377 DNA sequencer and DNA dye terminator cycle sequencing kit (Perkin Elmer, Oak Brook, IL). Multiple sequence alignments were carried out with the CLUSTAL W program version 1.6 [Thompson et al., 1994] and phylogenetic analyses were performed using the Phylogeny Interference Package (PHYLIP) [Felsenstein, 1989]. Phylogenetic distances were determined using the DNADIST program and phylogenetic trees were constructed using NEIGHBOR of the PHYLIP package. Both programs were used in combination with the SEQBOOT procedure (100 sets).

Statistical analysis was performed using the SPSS-PC program (SPSS, Chicago, IL). To compare qualitative values between patients/blood donors with and without TTV infection the chi-square test with Yates' correction was used. Parametric tests (Fisher's exact test with two-times-two tables) and nonparametric tests (Mann-Whitney U test) were used to compare quantitative values. Values are expressed as mean ± SEM, unless otherwise stated. *P*-values less than 0.05 were considered to be statistically significant.

RESULTS

TTV Infection In Blood Donors

TTV DNA was found in 21 (7%) of the 284 blood donors and TTV prevalence did not differ significantly between donors with or without elevated ALT levels (Table I). Donors with TTV infection were older (mean 41 ± 3.6 years) compared to TTV negative donors (mean 35 ± 1.4 years), but this difference was not statistically significant ($P = 0.14$). There was also no significant sex distribution among TTV positive and negative donors (38% vs. 37% females).

TTV in Patients With Liver Diseases

The overall prevalence of TTV infection in the 169 patients with acute and chronic liver disease was 15% (26/169) and significantly higher compared to the frequency observed in blood donors ($P = 0.012$). However, no significantly different TTV prevalence was found between the different groups of patients studied (Table I). In liver transplant patients (n = 105), TTV DNA was detected before OLT in 18 (17%) and after OLT in 56 (53%). Of the 18 patients with pre-transplant TTV infection, 8 (2 with cryptogenic cirrhosis, 4 with alcohol-induced cirrhosis, and 2 with fulminant hepatic failure non-A-E) became negative, while 10 (56%) remained positive within the observation period of 1–72 months

TABLE I. Prevalence of TTV in Blood Donors and Patients With Liver Disease

Group	TTV DNA positive
Blood donors (n = 284)	21 (7%)
Normal ALT levels (<21 U/L) (n = 249)	17 (7%)
Elevated ALT levels (>30 U/L) (n = 35)	4 (11%)
Patients with acute and chronic liver diseases before and after OLT (n = 105)	
Cryptogenic cirrhosis	
Before liver transplantation (OLT) (n = 32)	4 (12.5%)
After OLT (mean 38 months) (n = 32)	19 (59%)
Alcoholic liver disease	
Before OLT (n = 56)	8 (16%)
After OLT (mean 38 months) (n = 56)	26 (46%)
Fulminant hepatic failure non-A-E	
Before OLT (n = 17)	6 (35%)
After OLT (mean 39 months) (n = 17)	11 (65%)
Patients with chronic hepatitis C before and after antiviral treatment	
Before antiviral therapy (n = 64)	8 (12.5%)
3 months after initiation of antiviral therapy (n = 64)	0
6 months after end of treatment (n = 64)	3 (5%)

(mean 23 months) after OLT. Interestingly, the finding of TTV DNA after OLT was not associated with clinical or histopathological features of acute or chronic hepatitis. Thus, of the 56 post-transplant TTV positive patients only 3 (5%) had histological evidence of graft hepatitis. In the TTV negative patients (n = 49) six (12%) developed graft hepatitis (p = n.s).

Out of 64 patients with chronic hepatitis C who had received antiviral therapy, eight (12.5%) were found to have TTV DNA in serum before treatment and six had TTV DNA also associated to the peripheral blood mononuclear cells. The mode of HCV transmission in the eight TTV co-infected patients was blood transfusion (n = 4) and sporadic (n = 4). Interestingly, all eight TTV co-infected patients became TTV DNA negative during the course of antiviral treatment. However, three suffered a relapse and became TTV positive 6 months after stopping treatment. Of the five patients with long-term TTV response, four had received IFNa/ribavirin combination therapy. Patients with TTV co-infection were not significantly different from patients with HCV infection alone with respect to age, ALT levels (74 vs. 64 U/L), hepatitis C viremia levels (6.5 vs. 7.4 MEq/mL), and histological grading or staging.

Figure 1 shows the phylogenetic analysis of TTV sequences derived from 12 of the 21 TTV positive blood donors and 20 of the 26 TTV positive patients with liver disorders in comparison to published TTV sequences. The distribution of TTV genotypes was not significantly different in blood donors and patients: TTV type 1 (G1): 50% and 35%; TTV type 2 (G2): 33% and 45%, TTV type 3 (G3): 8% and 0%, and TTV type 4 (G4): 8% and 15%, respectively (p = n.s). In one patient, an unclassified isolate was found (F6) which showed strong homology to an isolate described recently from Poland (AF060550) [Höhne et al., 1998].

DISCUSSION

In the present study, the frequency of TTV infection in 284 blood donors and in 169 patients with liver diseases of known as well as unknown aetiology was investigated. By means of nested PCR, TTV sequences were found in 7% of the healthy blood donors and no significant difference between groups with normal or elevated ALT levels. In this respect, the study confirms the high prevalence of TTV infections in blood donors. However, in the USA and UK only 1–2% of the blood donors were infected as compared to 12% in the Japanese population and 36% in Thailand [Charlson et al., 1998; Okamoto et al., 1998a; Simmonds et al., 1998; Tanaka H et al., 1998]. These differences could account for different geographical distribution of TTV infections. Differences in the sensitivity of the PCR methods used may be another explanation for these discrepancies considering the low serum TTV DNA levels, as well as the high heterogeneity and variability of TTV isolates [Okamoto et al., 1998a; Simmonds et al., 1998]. Indeed, in a recent paper using a new set of possibly more universal PCR primers for the detection of TTV DNA, a 92% prevalence of TTV infections in the general, healthy population in Japan was found [Takahashi et al., 1998]. The finding of a higher rate of TTV infections in older blood donors is in accordance with recent reports [Simmonds et al., 1998; Takahashi et al., 1998]. Age, therefore seems to be a relevant risk factor in acquiring TTV infection.

The prevalence of TTV infection was higher in patients with liver disease than in the blood donors (15% vs. 7%) but occurred independently of the etiology of the liver disease. The higher frequency of TTV infection in patients with fulminant hepatic failure before OLT as compared to patients with alcoholic cirrhosis can be readily explained by the exposure to large amounts of blood products, which had to be given the former patients in the pre-operative period. Interestingly, 44% of the pre-operative TTV positive patients became negative after OLT. These data are in contrast to findings in patients with chronic hepatitis C or GBV-C infection where most patients remained viremic after OLT [Wright et al., 1992; Berg et al., 1996, 1999]. The reason for this discrepancy remains unclear. It is possible that antibodies to TTV, possibly contained in the blood products these patients had received, may have abrogated the infection.

The rate of de novo infections after OLT, however, was remarkably high in all liver transplant patients with a range between 39% in patients with alcoholic liver disease and 53% in patients with fulminant hepatic failure. Most likely this can be explained by transfusion of TTV contaminated blood products in the peri-operative period. Nevertheless, TTV could have been present in the donor and was then transmitted with the graft. The observation that TTV viremia after OLT was not associated with graft hepatitis in 53 of the

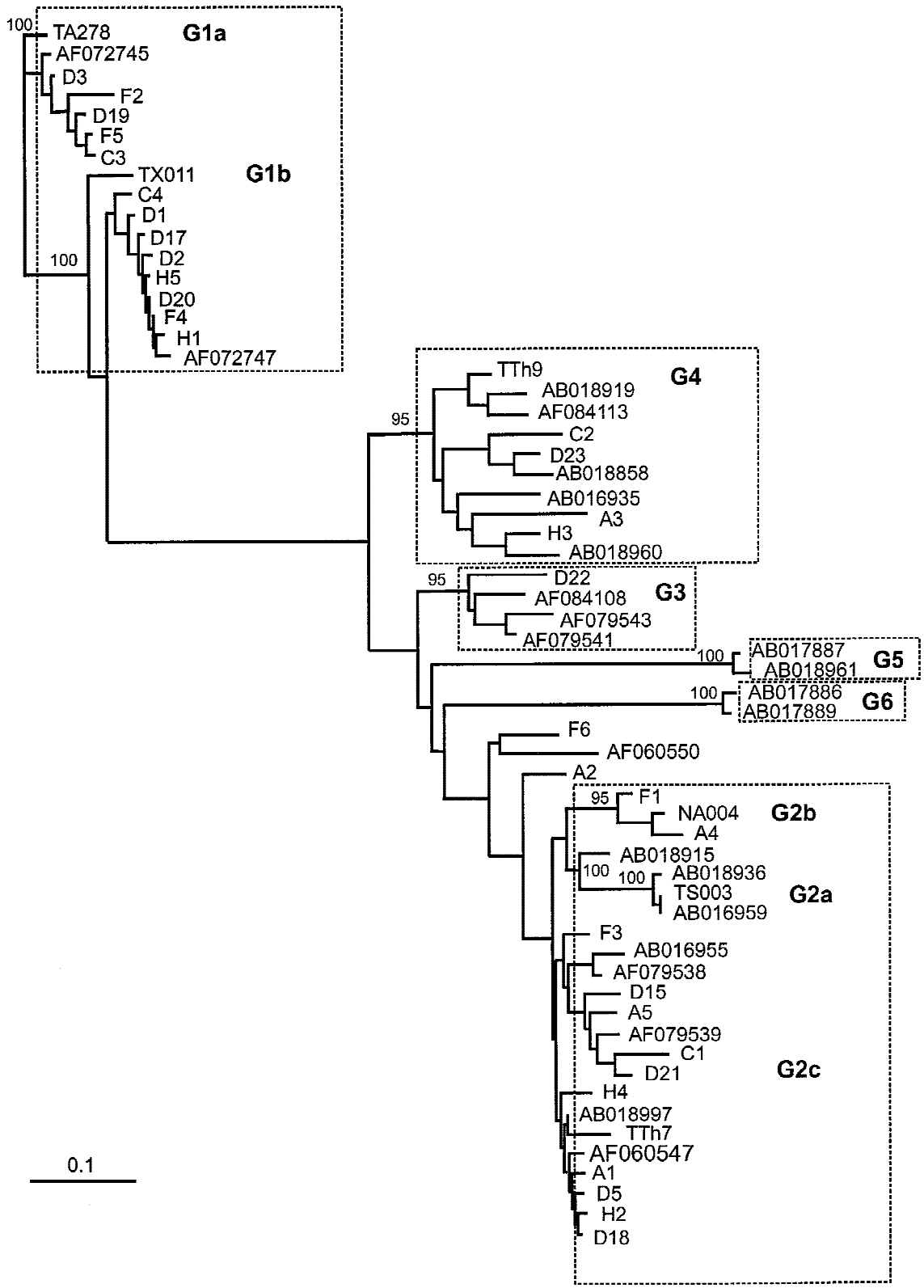

Fig. 1. Unrooted phylogenetic tree derived from alignments of TTV nucleotide sequences, nt position 1975 to 2165 according to GenBank accession number AB008394. Branch lengths are proportional to the evolutionary distances between sequences. The scale represents nucleotide substitutions per site. Following sequences were used for this analysis: TA278, TX011, TS003, and NA004 are prototypic sequences from Japan according to Okamoto et al. [1998a], representing subtypes G1a, G1b, G2a, and G2b, respectively. AF072745, AF072747, AF079538, AF079539, AF079541, and AF079543 are according to Simmonds et al. [1998]. AB016935, AB016955, AB016959, AB017886, AB017887, AB017889, AB018856, AB018897, AB018915, AB018919, AB018936, AB018960, and AB018961 are from Japan [Tanaka Y et al., 1998]. TTh7 and TTh9 are from Thailand [Tanaka H et al., 1998]. AF084108 and AF084113 are according to Viazov et al., 1999, and AF060547 and AF060550 are according to Höhne et al. [1998]. The present study included A1-A5, patients with alcoholic liver disease; C1-C4, patients with cryptogenic cirrhosis; F1-F6, patients with fulminant hepatic failure non-A-E; CHC1-CHC5, patients with chronic hepatitis C; and D1-D3, D5, D15, and D17-D23, blood donors (GenBank accession numbers AF10846 to AF10858). Only bootstrap values greater than 75% are indicated in support of each node.

56 TTV positive patients, as documented by histological follow-up analysis, is a strong argument against TTV being a hepatitis-inducing virus.

Analysis of the response to IFNa with or without ribavirin treatment in patients with chronic hepatitis C and co-infected with TTV clearly revealed that TTV is an interferon-sensitive virus with a universal response to this kind of therapy leading to a sustained clearence of TTV DNA from serum in five of the eight co-infected patients (63%). The comparison between TTV co-infected patients and patients with HCV alone showed that TTV co-infection did not affect severity of liver disease or hepatitis C viremia levels which is in accordance with a recent report from Naoumov et al. [Naoumov et al., 1998].

From alignments of all TTV nucleotide sequences available at present including the data of 32 positive patients/blood donors from Germany presented in this paper (see Fig. 1), TTV sequences were divided into at least six genomic groups (e.g., G1 to G6). These groups could each be divided further into subgroups (e.g., G1a, G1b, G2a, G2b, G2c). All TTV sequences derived from German patients or blood donors belonged to genotypes 1 to 4. One TTV isolate from a patient with fulminant hepatic failure (non-A-E) did not fit with any of the TTV genotypes reported so far, but showed strong homology to an isolate described recently in Poland (P/1; GenBank acc no. AF060550 [Höhne et al., 1998]). However, it is not known whether this genotype classification can be allocated to different genome parts or to full-length genomes. No significant differences in genotype distribution could be observed in patients with liver diseases or blood donors.

In conclusion, the high prevalence of TTV infection observed in our patients with parenteral risk factors is a strong argument in favour of virus transmission via blood and blood products. However, in view of the overall high prevalence in healthy individuals, the nonparenteral route of virus transmission cannot be ruled out. In this respect, the recent observation of excretion of TTV with feces is of interest [Okamoto et al., 1998b]. Most importantly, the present study provides no indication for a hepatitis-inducing capacity of TTV. One strong argument in favour of this concept is our observation that in the majority of OLT patients TTV infection was not accompanied by graft hepatitis.

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